

-57-

**Claims**

What is claimed:

1. A method to determine if a sample of cells contains dysplastic or carcinomic cells, the method comprising the steps of:

- 5                   a) contacting the sample with a solution of TCPP under conditions permitting binding of the TCPP to components of the abnormal precancerous or cancerous cells, if any are present;
- b) removing unbound TCPP from the sample; and
- c) detecting TCPP fluorescence in the sample, the presence of TCPP  
10   fluorescence being indicative that the sample contains dysplastic or carcinomic cells.

2. The method of claim 1, wherein the sample is selected from the group consisting of sputum samples, cervical swabs, bronchial washings, fine needle aspiration and core biopsies of thyroid and breast, bladder washings, urine, mouth washings, stool samples, blood or fractions thereof, lymph, cerebrospinal fluid, bone  
15   and bone marrow.

-58-

3. The method of claim 1, wherein the sample is fixed in a fixative selected from the group consisting of formaldehyde, methanol, ethanol, isopropanol and any combination thereof.

4. The method of claim 3, wherein the fixative is 95% ethanol.

5 5. The method of claim 1, wherein the sample is adhered to a solid support.

6. The method of claim 5, wherein the solid support is a microscope slide.

7. The method of claim 1, wherein the sample is suspended in a liquid medium.

8. The method of claim 1, wherein the solution of TCPP comprises the TCPP  
10 pre-dissolved in basified alcohol and diluted into a buffered aqueous solution.

9. The method of claim 8, wherein the solution of TCPP is buffered to a pH between about 5.8 and about 6.7.

10. The method of claim 8, wherein the solution further comprises one or more reagents that reduces background fluorescence, prevents oxidation of the  
15 TCPP, or prevents quenching of the TCPP fluorescence.

11. The method of claim 1, wherein the concentration of TCPP in the sample is between about 4 and about 100 µg/mL.

-59-

12. The method of claim 1, wherein the sample is contacted with the TCPP  
for between about 0.2 minute and about 2 hours.

13. The method of claim 1, wherein, during the contacting, the sample is  
maintained at a temperature between about 23°C and about 42°C.

5           14. The method of claim 5, wherein the TCPP fluorescence in the sample is  
detected visually.

15. The method of claim 5, wherein the TCPP fluorescence in the sample is  
detected with a slide reader.

10           16. The method of claim 7, wherein the TCPP fluorescence is detected with a  
fluorometric flow cytometer.

17. The method of claim 1, wherein the detecting step is performed between  
about 1 hour and about 24 hours after the removing step.

18. The method of claim 1, further comprising the step of determining the  
percentage of cells in the sample that are TCPP-fluorescent.

15           19. The method of claim 18, wherein samples comprising more than about  
1% fluorescent cells are categorized as containing abnormal precancerous or  
cancerous cells.

-60-

20. The method of claim 18, wherein the step of determining the percentage of cells in the sample that are TCPP-fluorescent comprises quantitating TCPP fluorescence intensity in the sample in a manner that correlates the fluorescence intensity with a percentage of cells in the sample containing TCPP.

5           21. The method of claim 20, wherein TCPP fluorescence is quantitated by contacting the sample with a detectable marker that binds to all cells in the sample, removing unbound detectable marker, and establishing a ratio of TCPP fluorescence and the amount of the detectable marker in the sample.

10           22. The method of claim 21, wherein the detectable marker is a fluorescent compound.

23. The method of claim 1, which further comprises, upon detecting TCPP fluorescence in the sample, characterizing the fluorescing cells for metaplasia, dysplasia or carcinoma.

15           24. The method of claim 23, wherein the characterizing comprises classifying the fluorescence intensity of fluorescent cells and correlating the fluorescence intensity with the metaplastic, dysplastic or carcinomic state of the cells.

25. The method of claim 23, wherein the characterizing comprises classifying the fluorescent cells for one or more morphological features selected from the group consisting of cell shape, cell size, clustering of cells, amount of degeneration of cells

-61-

or cell clusters, number of nuclei, size of nuclei, visibility of cell membrane and presence of nuclear debris, and correlating the morphological features with the metaplastic, dysplastic or carcinomic state of the cells.

26. The method of claim 23, wherein the characterizing comprises
- 5 classifying the fluorescent cells for fluorescence intensity and for one or more morphological features selected from the group consisting of cell shape, cell size, clustering of cells, amount of degeneration of cells or cell clusters, number of nuclei, size of nuclei, visibility of cell membrane and presence of nuclear debris, and correlating the fluorescence intensity and morphological features with the metaplastic,
- 10 dysplastic or carcinomic state of the cells.

27. The method of claim 26, wherein the total number of the morphological features and fluorescence intensity displayed by the fluorescent cells is used as a factor in characterizing the fluorescing cells for metaplasia, dysplasia or carcinoma.

- 15 28. The method of claim 26, wherein the pattern of morphological features and fluorescence intensity is used as a factor in characterizing the fluorescing cells for metaplasia, dysplasia or carcinoma.

29. The method of claim 23, wherein the fluorescent cells in the sample are compared with non-fluorescent cells from the same sample or from a second sample
- 20 from the same patient.

-62-

30. The method of claim 29, wherein the fluorescent cells are separated from the non-fluorescent cells by fluorometric flow cytometry.

31. A method of diagnosing a patient for early-stage cancer or a pre-cancerous condition of a selected tissue or organ, the method comprising:

- 5                   a) obtaining a sample of cells from the selected tissue or organ; and
- b) determining if the sample of cells contains abnormal precancerous or cancerous cells by the method of claim 1, a positive determination thereof being indicative of a positive diagnosis of early-stage cancer or a pre-cancerous condition of the patient's selected tissue or organ.

10               32. A method of prognosing a patient's response to a cancer therapy, the method comprising;

- a) prior to the therapy, performing the method of claim 1 on a sample of cells from the patient's tissue or organ being treated for the cancer;
- b) at intervals during the therapy and subsequent to the therapy, performing
- 15   the method of claim 1 on another sample of cells from the patient's tissue or organ being treated for the cancer; and
- c) determining if the percentage of abnormal pre-cancerous or cancerous cells in the samples tested during and subsequent to the therapy are reduced as compared

[illegible][illegible][illegible]

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99
0	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99

[illegible][illegible][illegible]

-64-

35. A method of detecting dysplastic or carcinomic cells in a selected target tissue of a patient, the method comprising the steps of:

a) introducing into the target tissue a solution of TCPP under conditions permitting binding of the TCPP to components of the dysplastic or carcinomic cells, if any are present;

b) removing unbound TCPP from the target tissue; and

c) detecting TCPP fluorescence in the cells of the target tissue, the presence of TCPP fluorescence therein being indicative that the target tissue contains dysplastic or carcinomic cells.

36. The method of claim 35, wherein the target tissue is selected from the group consisting of lung, breast, prostate gland, cervix, throat, bladder, oropharynx, skin, and gastrointestinal tract.

37. A method of making a TCPP solution, comprising dissolving the TCPP in an aqueous alcoholic solution comprising between about 50% and about 90% alcohol, at a pH between about 8.5 and about 12.0.

38. The method of claim 37, wherein the alcohol is isopropanol.

39. The method of claim 37, wherein the pH is adjusted with sodium bicarbonate or ammonium hydroxide.



-65-

40. The method of claim 37, wherein the TCPD dissolved is about 2 mg/mL or less.

41. A composition comprising TCPD dissolved in an aqueous alcoholic solution of about 50% to about 90% alcohol at a pH of between about 8.5 and about 12.0.

42. The composition of claim 41, wherein the concentration of TCPD in the solution is about 2 mg/mL or less.

43. The composition of claim 41, wherein the alcohol is isopropanol.

44. The composition of claim 41, having the pH adjusted with sodium bicarbonate or ammonium hydroxide.

45. The composition of claim 41, comprising 1 mg/mL TCPD in 50% isopropanol and 50 mM sodium bicarbonate.

46. A kit for detection of dysplastic or carcinomic cells in a sample, the kit comprising TCPD in a container and instructions for its use for detection of abnormal precancerous or cancerous cells in a sample.

47. The kit of claim 46, comprising the composition of claim 41.

